Studies on the Isolation and Genetic Nature of Specific Insecticide Resistance in Houseflies

Ansari, Jamil A.

Studies on the Isolation and Genetic Nature of Specific Insecticide Resistance in Houseflies
Jamil A. Ansari (Department of Zoology, Aligarh Muslim University, Aligarh, India) Received April 5, 1969. *Botyu-Kagaku* 34, 70, 1969).

9. DDT, ディールドリン抵抗性イエバエの分離とその遺伝的性質. Jamil A. Ansari (Department of Zoology, Aligarh Muslim University, Aligarh, India) 43. 4. 5. 受理

複数の殺虫剤に対して抵抗性のイエバエ *Musca domestica nebulo* をつくるため，DDT とディールドリンで交互に淘汰を30代行なった．その結果両薬剤に対して明らかに抵抗性のイエバエが得られた．この抵抗性イエバエに感受性のイエバエで戻し交雑を繰返し，DDT あるいはディールドリンで淘汰した結果，両殺虫剤にそれぞれ抵抗性の系統を得た．

両系統の交雑により，DDT 抵抗性は単一因子によるメンデル遺伝であり，ディールドリン抵抗性は複数の因子により遺伝され，明らかな分離を認められなかった．

Introduction

The failure of DDT to acheive effective housefly control due to the development of resistance has led to the introduction of some newer insecticides. But the housefly which has already developed resistance against DDT was able to develop resistance to subsequent insecticides which succeeded it, producing thereby the so-called 'Multiple insecticide resistant strain'. Occurrence of such a strain has been reported by Schoof *et al* (1951), Hess (1952), Keiding (1953) and Goodwin-Bailey *et al* (1954). from various parts of the world. Nearly everywhere the development of resistance has progressed to a marked degree, more or less at the verge of immunity. Such a development of multiple insecticide resistance by a single strain of housefly to a number of chemically unrelated compounds poses a serious problem for the insect control in general and the housefly control in particular with regard to the progress of human health and welfare. To overcome such a menacing problem a knowledge of the type of inheritance to a certain insecticide is very essential as it may offer valuable informations during control operations. The fact that predictions or interpretations of rates of development or regression of resistance are untenable without any understanding of the mode of inheritance or the character of resistance, is another aspect related in solving this problem. Furthermore, a quantitative analysis of the relative frequency of the genes for inheritance of resistance is obtained by the knowledge of the phenotypic response of the various genotypes to a certain insecticide.

There are enough published data on the inheritance of DDT and Dieldrin resistance in the housefly, but all are very divergent. Bruce and Decker (1950) reported a polygenic factor responsible for the hereditary transmission of DDT-resistance. Harison (1951) on the other hand found a single gene inheritance for DDT resistance. Keiding (1953) supported the view of Harison. A dominant gene for resistance to DDT has been reported in houseflies by Lichtwardt (1956). Dominant gene responsible for DDT-resistance have also been reported by Oshima and Hiroyoshi (1956). Milani (1957) has proposed that DDT-resistance in the housefly may be controled by a single gene which is partially dominant for the characteristic. There are also contrary reports on the inheritance of resistance to Dieldrin in the housefly. Resistance to Dieldrin has been shown to be monofactorial by Georghiou, March and Printy (1963), Lanna (1963), Milani (1963), Guneidy and Buswine (1964). Whereas Abdullah (1961), Rahman and Khan (1964) published results which show that inheritance of Dieldrin resistance is polygenic.

Under these circumstances revaluation, however, of certain of the study is very essential. It is, therefore, not unlikely that repitition of the study utilizing homogeneous strains of the housefly and a full range of closely spaced dosages, may yield more complete data which will necessitate reappraisal of the previous interpretations. With this purpose the present study has been undertaken. It deals with the development of a multiple resistant strain in the laboratory so as to provide material for the isolation of specific resistant strains for genetical studies.

Material and Method

The housefly, *Musca domestica nebulo* was used as the test insect. Multiple insecticide resistant strain was raised from the parent susceptible stock by the selection pressure of DDT and Dieldrin. The stock culture of the susceptible houseflies has never been exposed to any insecticide. All the strains utilized in the experiment were reared on milk soaked cotton at a temperature $28^\circ \pm 1^\circ C$ and humidity between 70~80%. The insecticides used were pure DDT and Dieldrin obtained from the Shell International Chemical Company, London. The formulations used were prepared in Acetone.

The experimental technique as adopted for the development of multiple resistant strain mainly consisted of topical application of the insecticide to the thorax of the houseflies and recording their percentage mortality after 24 hours. The 4-day old flies unmated of each sex were exposed to DDT and Dieldrin in alternate generations. The concentration of the insecticide was gradually increased to obtain a kill of 80~90%. In the 30th generation when resistance appeared to have fairly stabilized, LC_{50} value was determined with the help of log-concentration regression lines. For comparision susceptible strain was also similarly tested.

Isolation of specific resistant strain was carried out by a combination of back-cross and selective breeding. Multiple resistant flies were crossed with those of the susceptible, and the progeny was divided into two colonies. One of the colony was selected for resistance to DDT and the other for resistance to Dieldrin. After the selection pressure the surviving males from respective colonies were back-crossed with the susceptible females and the progeny was selected again. The process continued for six back generations and in each generation LC_{50} of the houseflies selected for resistance, together with their cross-tolerance was determined.

Inheritance studies were made by single pairs of reciprocal cross-mating of male and female parents of susceptible and isolated DDT and Dieldrin resistant strains. A portion of the F_1 generation thus obtained was inbred to produce F_2 generation and the rest were used to measure their response to graded doses of the insecticides. All the individuals of F_2 generation were tested for their level of resistance in the same manner. The concentrations used were sufficient to cover the complete range of susceptibility from the LC_{50} with the most susceptible strain to nearly an LC_{50} with the most highly resistant strain.

For convenience the various strains of the houseflies will be reffered to hereafter as S for susceptible strain, Multi-R, DDT-R and Dld-R respectively for Multiple, DDT and Dieldrin resistant strains.

Results and Discussion

Development of multiple resistant strain:

LC_{50} values of Multi-R and S houseflies have been determined and presented in the Table (1). Resistant ratio indicates that the process of development of resistance has greatly intensified by submitting the houseflies to DDT and Dieldrin in alternate generations. The houseflies have developed enough resistance to be utilized for experimental purposes.

Isolation of specific strains:

LC_{50} values of parents, hybrid and offspring from backcrosses after selection of hybrids with DDT and Dieldrin have been presented in the Table (1) and illustrated in Figures (1, 2, 3 and 4). The F_1 progeny of the reciprocal crosses between the Multi-R and S strain were identical with each other as regard to their DDT and Dieldrin resistance, which was intermediate between that of their parents. Either group of the hybrid progeny after being exposed to selection pressure from DDT to Dieldrin, the male survival was back-crossed with female susceptible strain giving two first back-groups under DDT-pressure and two first back-cross groups under Dieldrin-pressure. Since no significant difference could be found between each of the two groups in their susceptibility to DDT or Dieldrin, only one of the group in each case was choosen to produce further back-cross generations. In the sixth back-cross the colony DDT-R, selected for DDT, maintained

Table 1. Dosage mortality response of parents, hybrids and offsprings from back-crosses after selection of hybrids with DDT or Dieldrin.

Generation	Strain	Selection Pressure	DDT		Dieldrin	
			LC_{50}	R/N	LC_{50}	R/N
Parent	Susceptible		0.32		0.031	
Parent	Multi-R		170.00	531	2.55	82
F_1	♂R×♀S		51.0	159	1.9	61
	♀R×♂S		44.0	137	1.82	58
Ist Back Cross	♂(♂R×♀S)×♀S	DDT	36.0	112	0.58	18
	♂(♀R×♂S)×♀S	DDT	31.5	98	0.51	16
	♂(♂R×♀S)×♀S	Dieldrin	19.9	62	0.75	24
	♂(♀R×♂S)×♀S	Dieldrin	17.7	55	0.76	24
2nd Back Cross	♂(♂R×♂S)×♀S	DDT	17.5	54	0.48	15
	♂(♂R×♀S)×♀S	Dieldrin	15.5	48	0.65	20
3rd Back Cross	♂(♂R×♀S)×♀S	DDT	12.5	39	0.35	11
	♂(♂R×♀S)×♀S	Dieldrin	11.9	37	0.45	14
4th Back Cross	♂(♂R×♀S)×♀S	DDT	26.5	82	0.31	10
	♂(♂R×♀S)×♀S	Dieldrin	8.4	26	1.1	35
5th Back Cross	♂(♂R×♀S)×♀S	DDT	62.5	195	0.21	6
	♂(♂R×♀S)×♀S	Dieldrin	5.6	17	1.5	48
6th Back Cross	♂(♂R×♀S)×♀S	DDT	110.0	343	0.137	4
	♂(♂R×♀S)×♀S	Dieldrin	3.5	10	2.1	67

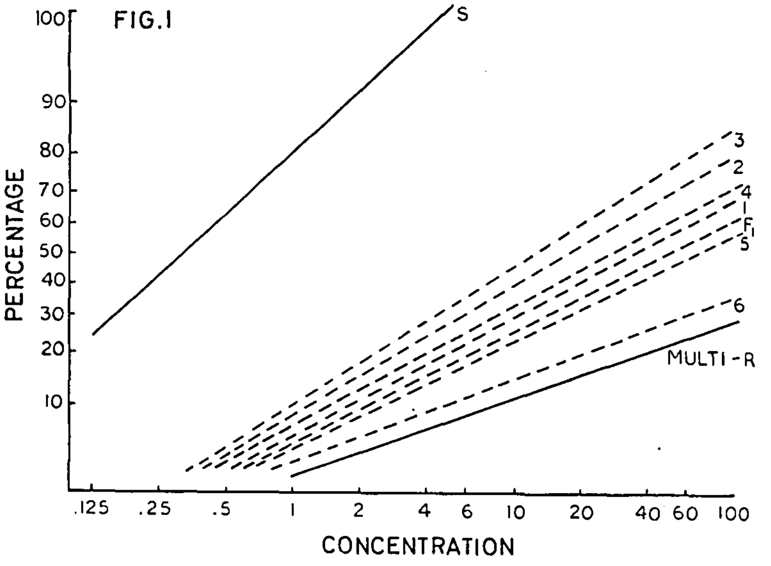

Fig. 1. Changes in the dosage-mortality response against DDT of the progeny from back crosses after selection of the hybrid with DDT.

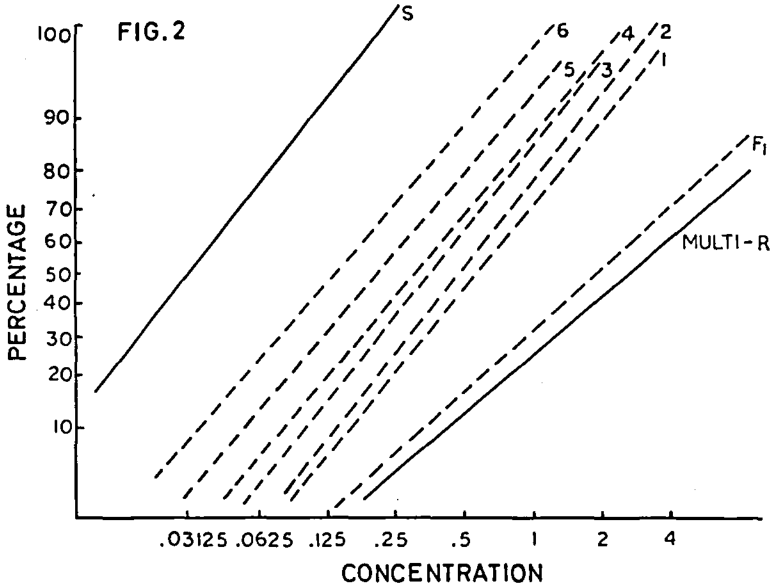

Fig. 2. Changes in the dosage-mortality response against Dieldrin of the progeny from back crosses after selection of the hybrids with DDT.

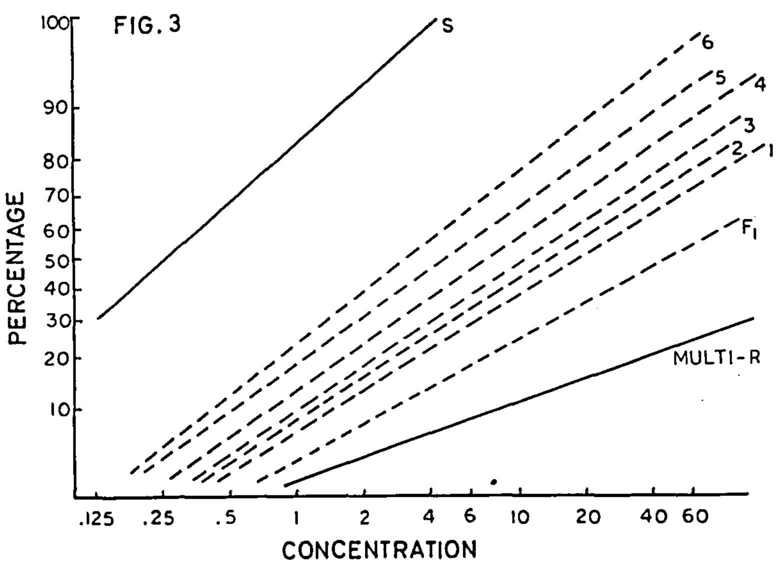

Fig. 3. Changes in the dosage-mortality response against DDT of the progeny from back crosses after selection of the hybrids with Dieldrin.

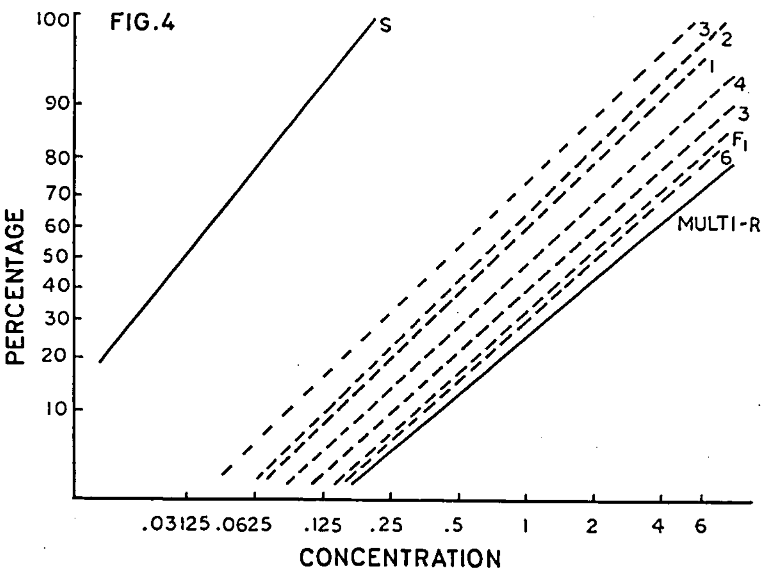

Fig. 4. Changes in the dosage mortality response against Dieldrin of the progeny from back crosses after selection of the hybrids with Dieldrin.

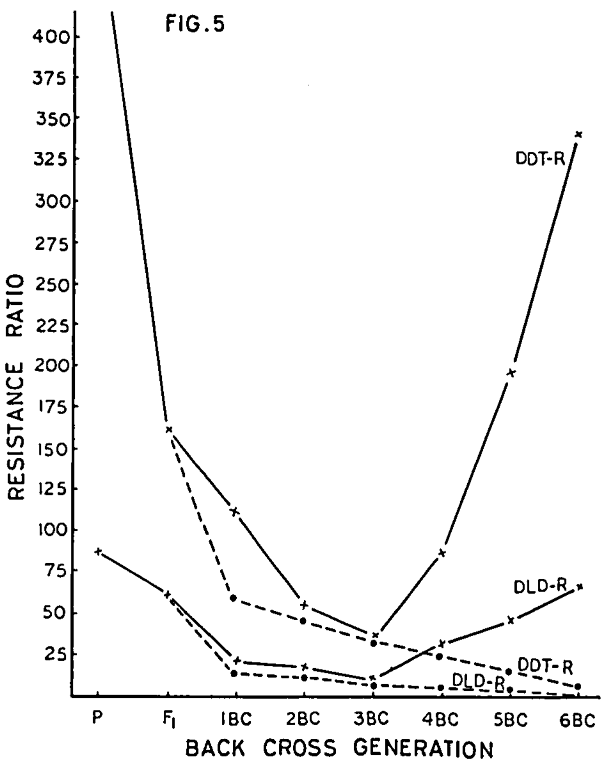

Fig. 5. Resistance pattern of the specific insecticide resistant strains and their cross-resistance characteristics.

its DDT-resistance in the strain; while the repeated back crossing with S-houseflies lowered Dieldrin resistance practically to normal (Fig. 5). Similarly the colony Dld-R, selected for Dieldrin, developed resistance against Dieldrin and at the same time its DDT-resistance (Fig. 5). Both DDT-R and Dld-R strains have developed significant resistance and their response have been found to be specific against those insecticides which were used for their selection. Any increase in their cross tolerance against other hydrocarbon insecticides and cyclodiene group of compounds could not be observed. After the selection procedure described DDT-R and Dld-R strains were maintained in isolation without further treatment.

Inheritance of resistance:

Summarized results of test on groups male and female in parental, F_1 and F_2 generations for DDT and Dieldrin resistance were given in Tables (2 and 3) and illustrated in Figures (6, 7, 8 and 9). There is no evidence for a sex-linked factor in inheritance either in the F_1 or the F_2 progenies of the crosses between male and female parents of susceptible or resistant origin. In view of the close agreement of the reciprocal crosses it is concluded that resistance is autosomal, being transmitted to the offspring by either parents. Therefore, the results of one of the reciprocal crosses have been selected and plotted for males and females separately.

Mortalities obtained with F_1 houseflies in the cross in which DDT-R parent was used show that resistance is partially dominant over susceptibility as the houseflies were slightly less resistant than the resistant parent and far more resistant than the susceptible ones (Figs. 6 and 7). However, the higher mortality in F_1 than the resistant parent indicated that the resistance is

Table 2. Percentage mortalities of the parental and their reciprocal crosses.

DDT (μg/fly)	Parent strain				♂S×♀DDT-R				♀S×♂DDT-R			
	Susceptible		DDT-R		F_1 hybrid		F_2 generation		F_1 hybrid		F_2 generation	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0.03125	16.6	7.1										
0.0625	26.6	14.1										
0.125	43.7	24.5										
0.25	49.1	39.9					5.2	2.3			4.8	3.5
0.5	73.3	51.7	3.2	2.4	6.8	4.5	15.1	10.1	7.2	5.2	14.5	12.2
1.0	81.6	81.3	7.1	5.3	9.5	8.0	23.5	21.3	10.1	9.2	24.4	24.5
2.0	91.5	89.6	9.3	6.7	19.1	10.3	52.4	40.5	18.2	11.5	45.6	39.4
4.0	100.0	96.8	12.5	9.7	22.5	18.5	56.1	45.5	21.5	19.4	57.2	42.6
6.0			16.9	13.5	27.9	23.2	63.5	47.2	28.5	24.2	64.5	50.0

Table 3. Percentage mortalities of the parental and their reciprocal crosses.

Dld. (μg/fly)	Parent strain				♂S×♀Dld-R				♀S×♂Dld-R			
	Susceptible		Dld-R		F_1 hybrid		F_2 generation		F_1 hybrid		F_2 generation	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0.015625	20.1	17.5										
0.03125	59.8	41.3					5.2	6.2			4.5	7.1
0.0625	82.7	67.5			10.0	7.2	16.5	10.5	11.2	8.5	15.2	11.2
0.125	100.0	89.6	3.1	2.3	29.9	25.7	31.2	32.5	26.8	24.1	30.5	28.4
0.25		100.0	6.2	4.6	42.5	34.4	61.5	40.5	45.1	36.4	55.6	41.2
0.5			15.9	9.1	70.1	60.1	90.0	78.2	68.5	58.2	88.1	75.4
1.0			33.7	24.3	90.1	71.2	96.2	85.3	85.3	75.4	95.4	84.2
2.0			55.4	41.6	96.2	79.3	100.0	89.1	92.4	80.5	100.0	90.1
4.0			85.3	59.5	100.0	90.4		90.3	100.0	91.3		95.5

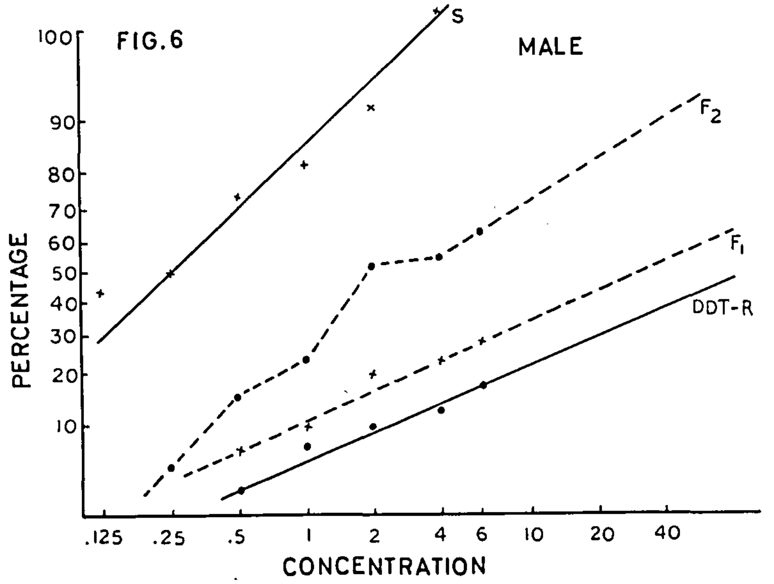

Fig. 6. Dosage-mortality regression lines for male flies of the cross ♂S×♀(DDT-R).

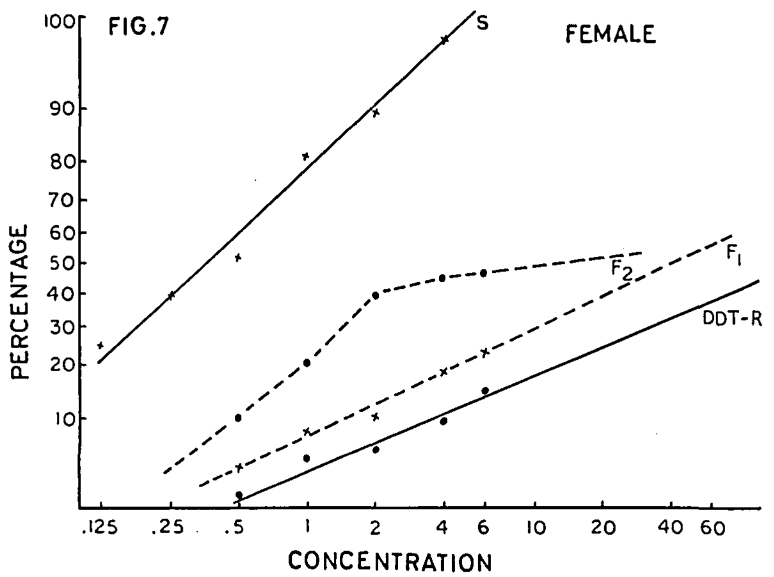

Fig. 7. Dosage-mortality regression lines for female flies of the cross ♂S×♀(DDT-R).

not completely dominant. The test with the F_2 is almost intermediate between the susceptible and resistant parents. The pattern of the regression lines for male and female parents, F_1 and F_2 remained essentially the same, with slight inflation towards greater susceptibility in the males (Fig. 6). It is not clear whether this is due to reduced vigor in the male offspring or some influence of rearing media. The approximate 1:2:1 (1 susceptible homozygote, 2 heterozygote, 1 resistant homozygote) segregation to be expected in monofactorial inheritance is indicated by the

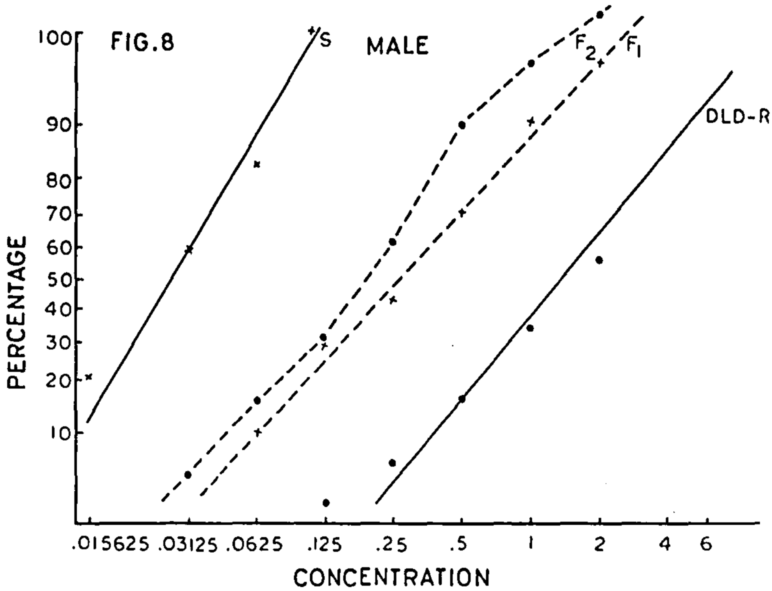

Fig. 8. Dosage-mortality regression lines for male flies of the cross ♂S×♀(Dld-R).

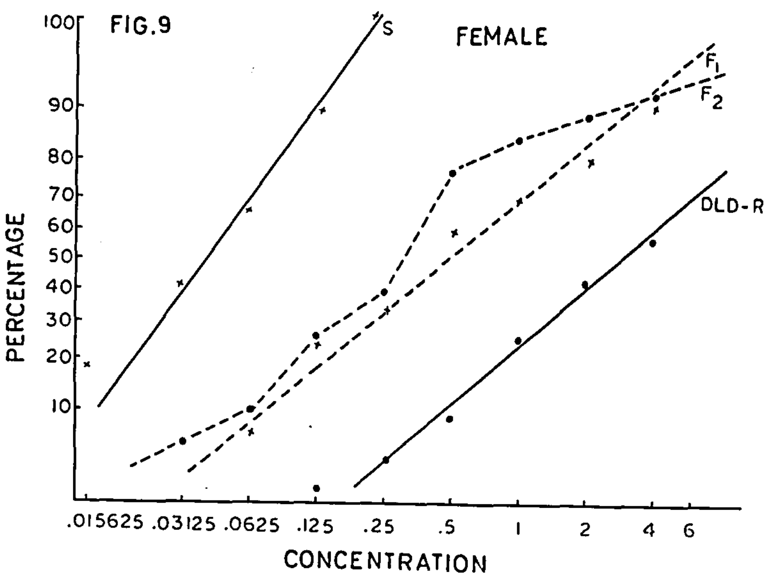

Fig. 9. Dosage-mortality regression lines for female flies of the cross ♂S×(♀Dld-R).

result. It is concluded, therefore, that the DDT resistance is in agreement with a single gene hypothesis for inheritance of resistance.

The regression lines obtained in the F_1 generation of the cross in which Dld-R parent was used show a response intermediate to that of the parents (Figs. 8 and 9). It is indicated that Dieldrin resistance is neither dominant nor recessive. The dosage-mortality tests run in F_2 generation and the figures obtained show that in this case also the gene causes an intermediate degree of resistance but at a slightly lower level than the F_1 generation. There is no segregation in the F_2 generation which indicates that the progeny consists of varying genotypes. The presence of many genes is further proved by the wider variance of resistance to the insecticides which is reflected in F_2 regression lines presenting greater slopes as compared to the F_1 progeny.

Summary

A multiple insecticide resistant strain, in the housefly *Musca domestica nebulo*, has been developed by selection pressure of DDT and Dieldrin in alternate generations under laboratory condition so as to provide material for isolation of the different forms of resistance. Tests carried out by repeated back crossing combined with selection pressure from DDT and Dieldrin produced specific DDT and Dieldrin resistant strains. Further, information on the mode of inheritance of DDT and Dieldrin resistance was obtained by determining the relative toxicity of the insecticide to the resistant and susceptible strains; the F_1 progeny of the reciprocal crosses; F_2 generation by inbreeding of the F_1 generation. The experiment with DDT resistant houseflies demonstrated a population segregating in 1:2:1 Mendalian ratio which indicated that resistance to DDT in the housefly is inherited as major single factor. While the experiment in which Dieldrin houseflies were involved proved to be multifactorial inheritance as the F_1 and F_2 generations were both intermediate between their parents without showing any segregation.

Acknowledgement: The author is grateful to Prof. S. M. Alam, Head, Department of Zoology, for providing facilities and encouragement during the course of the present study.

References

Abdullah, M.: Inheritance of Dieldrin resistance in the housefly. *Jour. Hered.* 52 (4), 179~182 (1961).

Bruce, W. N., and G. C. Decker.: Housefly tolerance for insecticides. *Soap and Sanitary Chemicals.* 26(3), 122~125, 145~147 (1950).

Busvine, J. R.: The insecticide potency of BHC and chlorinated cyclodiene compounds and the significance of resistance to them. *Bull. Entomol. Research*, 55, 271~288 (1964).

Georghiou, G. P., March, R. B. and Printy, G. E.: A study of the genetics of Dieldrin resistance in the housefly, *Musca domestica* L. *Bull. Wld. Hlth. Org.* 29, 155~165 (1963).

Goodwin-Bailey, K. F., and M. Davies.: Occurrence of Dieldrin resistance in wild *Musca domestica* L. in England. *Nature*, 173, 216~217 (1954).

Harison, C. M.: Inheritance of resistance to DDT in the housefly, *Musca domestica* L. *Nature*, 167, 855~856 (1951).

Hess, A. D.: The significance of insecticide resistance in vector control programms. *Am. J. Trop. Med. Hyg.* 1, 371~388 (1952).

Keiding, J.: Development of resistance in the field and studies of inheritance. *Trans. 9th Intern. Congr. Entomol.* 2, 340~345 (1953).

Lanna, M. T.: The inheritance of insecticide resistance. *Proceedings XI th Internat. Congress* of *Genetics.* 1, 254 (1963).

Lichtwardt, E. T.: Genetics of DDT-resistance of an inbred line of housefly. *Jour. Hered.* 47 (1), 11~16 (1956).

Milani, R.: Genetic research on the resistance of insects to the action of toxic substances. Suppl. C, Oct. 1957. *Inform. Circ. Probl., Wld. Hlth. Org. Div. Environm. Sanit.* (1957).

Milani, R.: The genetics of insect resistance to insecticides. *Proceedings XIth Internat. Congress* of *Genetics.* 1, 255 (1963).

Oshima, C., and T. Hiroyoshi.: Genetic studies of resistance to DDT and Nicotine sulphate in *Drosophila virilis. Botyu-Kagaku.* (*Bull. Inst. Insect. Control*) 21, 65~70 (1956).

Rahman, J. S., and N. H. Khan.: Inheritance of Dieldrin resistance in *Musca domestica nebulo. Botyu-Kagaku.* (*Bull. Inst. Insect. Control*) 29, 19~21 (1964).

Schoof, H. F., R. E. Siverly, and J. H. Coffey.: Dieldrin as a chemical control material on community fly control programs. *J. econ. Ent.* 44, 803~807 (1951).